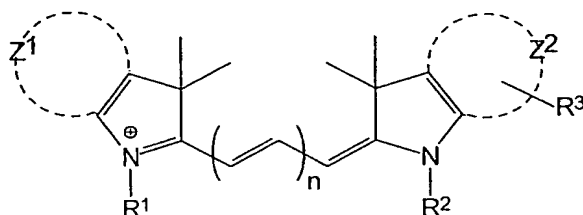


Claims

1. A matched set of fluorescent dyes comprising at least two different fluorescent dyes of formula (I):

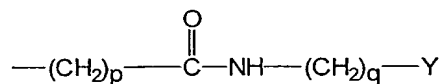


(I)

wherein n is different for each said dye and is 1, 2, or 3;

Z^1 and Z^2 independently represent the carbon atoms necessary to complete a phenyl or naphthyl ring system;

one of groups R^1 and R^2 is the group:



where Y is a target bonding group;

remaining group R^1 or R^2 is selected from $-(CH_2)_4-W$ or $-(CH_2)_r-H$;

group R^3 is hydrogen, except when either R^1 or R^2 is $-(CH_2)_r-H$, in which case R^3 is W ;

W is selected from sulphonic acid and sulphonate;

p is an integer from 3 to 6;

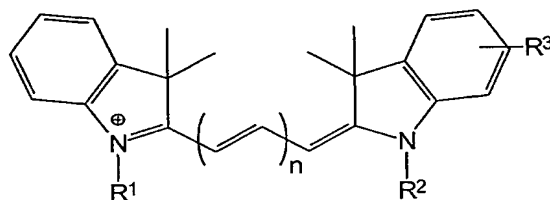
q is selected to be 2 or 3; and

r is an integer from 1 to 5;

and their salts;

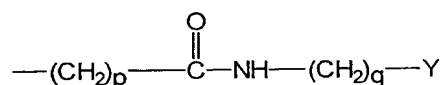
characterised in that when n of two of said dyes differs by + 1, one of p , q and r of said two dyes differs by -1.

2. A matched set of fluorescent dyes comprising at least two different fluorescent dyes of formula (II):



(II)

wherein n is different for each said dye and is 1, 2, or 3;
one of groups R^1 and R^2 is the group:



15

where Y is a target bonding group;

remaining group R^1 or R^2 is selected from $-(CH_2)_4-W$ or $-(CH_2)_r-H$;

group R^3 is hydrogen, except when either R^1 or R^2 is $-(CH_2)_r-H$, in which case R^3 is W ;

W is selected from sulphonic acid and sulphonate;

p is an integer from 3 to 6;

q is selected to be 2 or 3; and

r is an integer from 1 to 5;

and their salts;

characterised in that when n of two of said dyes differs by + 1, one of p , q and r of said two dyes differs by -1.

3. A matched set according to claim 1 or claim 2 comprising at least two different fluorescent dyes according to formula (I) or (II) in which:

n is selected to be 1 or 2;

p is selected to be 4 or 5;

q is selected to be 2 or 3; and

r is selected to be 1, 2 or 3.

4. A matched set according to any of claims 1 to 3 wherein said
5 target bonding group Y in each dye of the set of dyes is the same and is
selected from a maleimido group and an iodoacetamido group.

5. A matched set according to claim 4 wherein in each said dye Y is a
maleimido group.

10

6. A matched set according to any of claims 1 to 5 wherein said salts
are selected from K^+ , Na^+ , NH_4^+ , R_3NH^+ and R_4N^+ where R is C_1 to C_4
alkyl.

15 7. A matched set of dyes according to any of claims 1 to 6 selected
from:

Set 1

20 1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-2-
[(1*E*,3*E*)-3-(1-ethyl-3,3-dimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-
ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound I); and
1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-
3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(1,3,3-trimethyl-5-sulpho-1,3-dihydro-2*H*-
25 indol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound II);

Set 2

1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-2-
30 [(1*E*,3*E*)-3-(1-propyl-3,3-dimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-
ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound III); and

1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(1-ethyl-3,3-trimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound IV);

5 Set 3

1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-2-[(1*E*,3*E*)-3-(1-ethyl-3,3-dimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound I); and

10 1-(5-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxopentyl)-3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(1-ethyl-3,3-trimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound V);

Set 4

15

1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-2-[(1*E*,3*E*)-3-(3,3-dimethyl(1-sulpho-butyl)-1,3-dihydro-2*H*-indol-2-ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound VI); and

20 1-(5-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxopentyl)-3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(3,3-dimethyl-(1-sulpho-butyl)-1,3-dihydro-2*H*-indol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound VII).

Set 5

25 1-(6-{[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]amino}-6-oxohexyl)-2-[(1*E*,3*E*)-3-(1-ethyl-3,3-dimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound VIII); and

30 1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(1-ethyl-3,3-trimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound IV); and

Set 6

1-(6-[[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]amino]-6-oxohexyl)-2-
[(1*E*,3*E*)-3-(3,3-dimethyl(1-sulpho-butyl)-1,3-dihydro-2*H*-indol-2-ylidene)prop-
5 1-enyl]-3,3-dimethyl-3*H*-indolium (Compound IX); and
1-(6-[[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino]-6-oxohexyl)-3,3-
dimethyl-2-[(1*E*,3*E*,5*E*)-5-(3,3-dimethyl-(1-sulpho-butyl)-1,3-dihydro-2*H*-indol-
2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound X).

10 8. A method for labelling a mixture of proteins in a sample wherein each
of said proteins contains one or more cysteine residues, said method
comprising:

- i) adding to an aqueous liquid containing said sample a fluorescent dye
selected from a matched set of fluorescent dyes wherein each said dye
15 contains a target bonding group that is covalently reactive with said proteins;
and
ii) reacting said dye with said proteins so that said dye labels said
proteins;
characterised in that all available cysteine residues in said proteins are
20 labelled with said dye.

9. A method according to claim 8 wherein said fluorescent dye is a
cyanine dye.

25 10. A method according to claim 9 wherein said cyanine dye contains a
sulphonic acid or sulphonate group.

11. A method according to any of claims 8 to 10 wherein said target
bonding group is selected from a maleimido group and an iodoacetamido
30 group.

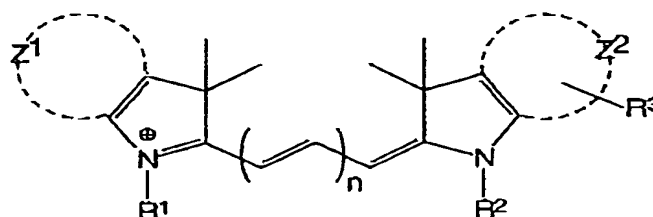
12. A method according to claim 8 further comprising prior to step i), the
step of treating the protein with a reductant.

13. A method according to claim 8 wherein said dye is used in a range of 5 to 200nmol of dye per 50µg of protein.

14. A method according to claim 8 wherein said labelling is performed at a pH in the range from 6.0 to 9.0.

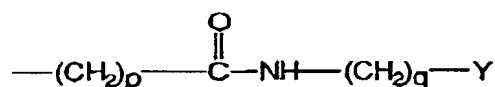
15. A method for labelling one or more proteins in a sample, the method comprising:

i) adding to a liquid sample containing said one or more proteins a fluorescent dye selected from a matched set of fluorescent dyes each dye in said set having the formula (I):



(I)

wherein n is different for each said dye and is 1, 2, or 3;
Z¹ and Z² independently represent the carbon atoms necessary to complete a phenyl or naphthyl ring system;
one of groups R¹ and R² is the group:



where Y is a target bonding group;
remaining group R¹ or R² is selected from -(CH₂)₄-W or -(CH₂)_r-H;
group R³ is hydrogen, except when either R¹ or R² is -(CH₂)_r-H, in which case R³ is W;
W is selected from sulphonic acid and sulphonate;
p is an integer from 3 to 6;
q is selected to be 2 or 3; and

r is an integer from 1 to 5;

and their salts;

characterised in that when n of two of said dyes differs by +1, one of p, q and r of said two dyes differs by -1; and

- 5 ii) incubating said dye with said sample under conditions suitable for labelling said one or more proteins.

16. A method according to claim 15 wherein each of Z^1 and Z^2 represents the carbon atoms necessary to complete a phenyl ring system.

10

17. A method according to claim 15 or claim 16 wherein:

n is selected to be 1 or 2;

p is selected to be 4 or 5;

q is selected to be 2 or 3; and

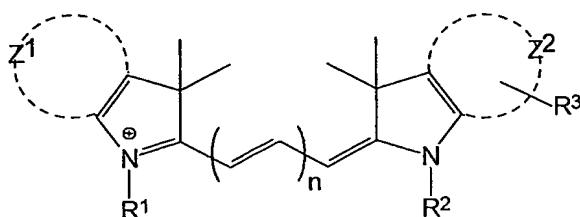
15 r is selected to be 1, 2 or 3.

18. A method according to any of claims 15 to 17 wherein said target bonding group Y is selected from a maleimido group and an iodoacetamido group.

20

19. A kit comprising a matched set of fluorescent dyes comprising at least two different fluorescent dyes having the formula (I):

25

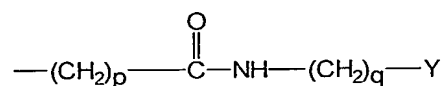


(I)

wherein n is different for each said dye and is 1, 2, or 3;

Z^1 and Z^2 independently represent the carbon atoms necessary to complete a phenyl or naphthyl ring system;

one of groups R^1 and R^2 is the group:



where Y is a target bonding group;

remaining group R^1 or R^2 is selected from $-(CH_2)_4-W$ or $-(CH_2)_r-H$;

group R^3 is hydrogen, except when either R^1 or R^2 is $-(CH_2)_r-H$, in which case R^3 is W ;

W is selected from sulphonic acid and sulphonate;

p is an integer from 3 to 6;

q is selected to be 2 or 3; and

r is an integer from 1 to 5;

and their salts;

characterised in that when n of two of said dyes differs by + 1, one of p ,

q and r of said two dyes differs by -1.